PHOTOSYNTHETIC BIOFILMS IN CARLSBAD CAVERN: USE OF *IN SITU* SPECTROPHOTOMETRY AND DNA ANALYSIS TO EXPLORE INFLUENCE OF LIGHTING AND SUBSTRATE CONDITIONS ON GROWTH.

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Photosynthetic communities of microorganisms including algae and cyanobacteria, termed "lampenflora", have been proliferating in Carlsbad Cavern and other show caves worldwide since the adoption of artificial lighting systems. These suites of organisms prove a detriment to the aesthetics of the cave and can physically degrade the underlying speleothems on which they grow [1]. With the goal of curbing this growth, the National Park Service recently modernized the lighting in Carlsbad Cavern to an LED system that allows for a range of adjustment of color temperature and intensity. This functionality of the new system was used to lower the color temperature of the lights to a range that should be less conducive to photosynthesis.

Assessing the response of these biofilms without causing damage to the underlying speleothems warrants the use of non-destructive analytical methods. This study combined high-throughput DNA sequence analysis of the lampenflora communities with quantitative and semi-quantitative visual assessment of biofilm growth. Using a variety of technologies to examine microbial growth in the nutrient limited cave environment holds relevance to novel application of technologies to the search for extant life on other planets [2].

Lampenflora response to decreased color temperature output in the new LED system was monitored with a handheld reflected-light spectrophotometer. This device was used to correlate change in color to relative change in photosynthetic cell density. 16S and 18S rRNA gene amplicon sequencing provided data on microbial community composition.

The low biomass conditions at many of the study locations proved challenging for recovery of material for sequencing. Despite this, the taxonomy and relative abundance data indicated some variation in biofilms based on the host substrate. Different members of the photosynthetic community were observed in the biofilms, including green algae *Chlorophyta*, as well as several species of cyanobacteria. Community composition at the study sites appeared to shift over time, particularly for photosynthetic taxa.

The spectrophotometric data did not indicate the expected trend of increasing levels of growth over time, where instead some study sites saw decreased growth. There was minimal difference in growth rates at lower color temperature lit sites versus higher, which is was not expected. In terms of cave management, this may indicate that the lowered color temperature is not sufficient for reduction of lampenflora growth and additional removal methods may be necessary.

References

[1] J. Mulec, and G. Kosi, Lampenflora algae and methods of growth control (2009) *Journal of Cave and Karst Studies* 71, 109–115. [2] Boston P.J. et al. (2001) *Astrobiology*, *1*, 25-55.